Substance:	**Benzoic acid, 2-hydroxy-, Mono-C14-18 Alkyl Derivatives, Calcium Salts**
Summary prepared by:	**Petroleum Additives Panel Health & Environmental Research Task Group**
Date of last update:	**December 2006**
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1.0 Physico-chemical Data

1.1 Water Solubility

Robust Summary 14-WaterSol-1

Test Substance	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts. (Referred to in the final submission as AI-43)
Method	
Method/Guideline followed	Water solubility and n-octanol/water partition coefficient were estimated in accordance with guidelines from the UK Health and Safety Executive, the UK Competent Authority for the notification of new substances.
Test Type	Water solubility and partition coefficient (n-octanol/water) estimated based on ultraviolet absorption of aqueous solutions used as an approximate indicator of the concentrations of aromatic rings in water.
GLP (Y/N)	Not specified
Year (Study Performed)	1991
Test Material Solvent	Octanol, hexane and water
Methods	The OECD Shake flask method was determined to not be appropriate for this test material because it is a complex mixture. The test material contains aromatic rings, which absorb strongly in the ultraviolet region. The UV absorptions of aqueous solutions of the test material were, therefore, used as an approximate indicator of the concentrations of those components in water. The solubility of the test material in octanol was examined by addition of the test material to a quantity of octanol. The solubility of the aromatic components in water was determined, in duplicate, by shaking 1.2 g of test material with 10 mL of water for approximately 10 seconds by hand, immersing at 20 ^{o}C for 28 hours then centrifuging at 20^{o}C. The layers were separated using a separatory funnel and the aqueous layer was analyzed directly by UV-visible spectrophotometry with standard solutions of the test material in hexane. The solution was not equilibrated for a longer period of time due to concerns regarding test material stability.
Results	The concentration of the aromatic components in water was estimated to be approximately 9 mg/L. This value is an approximation because it assumes that the extinction coefficient for the test material standard is the same as that for the material dissolving in the water. That will not be the case, as some components of the test material will dissolve preferentially to others. Also the mineral oil component of the test material, which was not quantified in the analysis, might

	make some contribution to UV absorption resulting in a possible over estimate of the aromatic component of the aqueous solution. It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L. 4.49 g of test material was added to 2.33 g of octanol. A homogeneous solution was formed with a total volume of 7 mL. The result indicates that the solubility in octanol is greater than 0.64 g/mL. Although the aqueous solution will not contain the components of the test material in the same ratio as the hexane standard, the results allow an estimate to be made of the log P_{ow} as follows: $P_{ow} > 0.64/9 \times 10^{-6}$ or $>7 \times 10^{4}$ Log $P_{ow} > 4.9$ It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L. Based on the highest water solubility value of 90 mg/L, the log P_{ow} is > 3.9. A water solubility value of 0.9 mg/L equates to a log P_{ow} of > 5.9.
Conclusions	Log P_{ow} > 3.9, allowing for a possible ten fold error in the determination of the solubility of the test material in water. The solubility of the test material in water was approximately 9 mg/L (0.9-90 mg/L) for those species containing a chromophore.
Data Quality	Reliable without restriction.
References	Confidential business information.
Other	Updated: 11/21/2003

Robust Summary 14-WaterSol-2

Test Substance	
CAS #	114959-46-5 (Test Campaign 2 AI-28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C>13 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts Test material is a complex mixture. Because the various components have different limits of solubility the solubility of the mixture is dependent on loading rate.
Method	
Method/Guideline followed	The water solubility of this test material was determined according to the principles of the EC Test A6 guideline (Official Journal of the European Communities, 1992).
Test Type	Water Solubility
GLP (Y/N)	Yes
Year (Study Performed)	1996
Methods	Tests of the solubility of the test material were carried out in several sets. Loading rates of approximately 1, 10 and 100 g/L were utilized. Stirring times ranged from 6 to 96 hours. The typical procedure for a loading rate of 1 g/L was as follows: Approximately 0.10 g of test material was added to each test flask, 100 mL of water was added to each flask, the flasks were stoppered and placed in a water bath (20^{o}C). Each flask was stirred with a magnetic stirrer for the test period. The vortex extended two-thirds down the depth of the vessel. At the end of the incubation period the flasks were removed and allowed to settle. 25-30 mL of the solution was placed in a centrifuge tube and centrifuged at 3000 rpm for 30 minutes at 30^{o}C. Two analytical approaches were used to determine solubility, total; carbon analysis and UV spectrophotometry. Total carbon analysis provided information about the sum of all carbon containing materials dissolved in water. UV spectrophotometry essentially determined only the calcium alkyl salicylate dissolved in water. Any inorganic calcium salts or mineral oil present in the aqueous phase were expected to have a low or essentially no UV absorbance. Since the test material contains calcium carbonate, inorganic carbon content was measured to determine whether the test material was physically stable in the water phase. Calcium content was also measured to establish whether any dissociated calcium salts were present.
Results	Total carbon analysis indicated that 24 hour vigorous stirring was sufficient to achieve equilibration. The mean values for the 1 g/L loading rate, after 24, 48 and 96 hours of vigorous mixing, were 20, 21 and 21 mg carbon/L, although the 96-hour values were variable (21$\pm$ 15 mg carbon/L). Mean values for the 100 g/L loading rate after 24, 48 and 96 hours of vigorous mixing were 65, 69 and 71

	mg carbon/L. Solubility was dependent on loading rate. The mean carbon content at 1 g/L and 100 g/L loading rates between 24 and 96 hours of stirring were 20 ± 11 mg and 69 ± 4 mg carbon/L. Previous studies have shown that the mineral oil component of the test material contributes 2.0 ± 1.0 mg carbon/L. The test material has a calculated percent carbon content of 67%; the concentration of components of the test material in the aqueous phase were 30 ± 16 mg/L and 103 ± 6 mg/L at 1 and 100 g/L loading rates respectively. The solubility of the test material determined by UV analysis was in broad agreement (with a few low values). Inorganic carbon and calcium analysis indicated that the test substance was physically stable in water.
Conclusions	The concentration of components of the test material in the aqueous phase were 30 ± 16 mg/L and 103 ± 6 mg/L at 1 and 100 g/L loading rates respectively.
Data Quality	Reliable without restriction.
References	Confidential business information.
Other	Updated: 11/27/2003

1.2 Partition Coefficient

Robust Summary 14-Log Kow-1

Test Substance	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts. (Referred to in the final submission as AI-43)
Method	
Method/Guideline followed	Water solubility and n-octanol/water partition coefficient were estimated in accordance with guidelines from the UK Health and Safety Executive, the UK Competent Authority for the notification of new substances.
Test Type	Water solubility and partition coefficient (n-octanol/water) estimated based on ultraviolet absorption of aqueous solutions used as an approximate indicator of the concentrations of aromatic rings in water.
GLP (Y/N)	Not specified
Year (Study Performed)	1991
Test Material Solvent	Octanol, hexane and water
Methods	The OECD Shake flask method was determined to not be appropriate for this test material because it is a complex mixture. The test material contains aromatic rings, which absorb strongly in the ultraviolet region. The UV absorptions of aqueous solutions of the test material were, therefore, used as an approximate indicator of the concentrations of those components in water. The solubility of the test material in octanol was examined by addition of the test material to a quantity of octanol. The solubility of the aromatic components in water was determined, in duplicate, by shaking 1.2 g of test material with 10 mL of water for approximately 10 seconds by hand, immersing at 20 ^{o}C for 28 hours then centrifuging at 20^{o}C. The layers were separated using a separatory funnel and the aqueous layer was analyzed directly by UV-visible spectrophotometry with standard solutions of the test material in hexane. The solution was not equilibrated for a longer period of time due to concerns regarding test material stability.
Results	The concentration of the aromatic components in water was estimated to be approximately 9 mg/L. This value is an approximation because it assumes that the extinction coefficient for the test material standard is the same as that for the material dissolving in the water. That will not be the case, as some components of the test material will dissolve preferentially to others. Also the mineral oil component of the test material, which was not quantified in the analysis, might make some contribution to UV absorption resulting in a possible over estimate of

	the aromatic component of the aqueous solution. It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L. 4.49 g of test material was added to 2.33 g of octanol. A homogeneous solution was formed with a total volume of 7 mL. The result indicates that the solubility in octanol is greater than 0.64 g/mL. Although the aqueous solution will not contain the components of the test material in the same ratio as the hexane standard, the results allow an estimate to be made of the log P_{ow} as follows: $P_{ow} > 0.64/9 \times 10^{-6}$ or $>7 \times 10^{4}$ Log $P_{ow} > 4.9$ It was estimated that the true water solubility of the aromatic components of the test material lies between 0.9 and 90 mg/L. Based on the highest water solubility value of 90 mg/L, the log P_{ow} is > 3.9. A water solubility value of 0.9 mg/L equates to a log P_{ow} of > 5.9.
Conclusions	Log P_{ow} > 3.9, allowing for a possible ten fold error in the determination of the solubility of the test material in water. The solubility of the test material in water was approximately 9 mg/L (0.9-90 mg/L) for those species containing a chromophore.
Data Quality	Reliable without restriction.
References	Confidential business information.
Other	Updated: 11/21/2003

2.0 Environmental Fate and Pathways

2.1 Biodegradation

Robust Summary 14-Biodeg-1

Test Substance	
CAS #	114959-46-5 (test campaign 1 AI-43)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
Method	
Method/Guideline Followed	Similar to ISO (1997) Headspace CO_2 Biodegradation Test with modifications recommended by CONCAWE
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1997
Contact time (units)	56 days
Test apparatus	160 mL capacity serum bottles
Inoculum	Activated sewage sludge from a domestic wastewater treatment plant and soil filtrate were collected. Sieved soil, activated sludge and yeast extract were added to 2 liters of mineral salts medium (OECD) and mixed. Eight adaptation cultures were prepared. Each flask was dosed with the test material (4 mg/L as carbon). The test substance was added on a glass fiber filter. The flasks were then closed with foam stoppers and incubated at 20^{o}C while shaking at 120 rpm. On days 7 and 11, any evaporation loss was made up with distilled water, the pH adjusted, if necessary, to between 7.2 and 7.6, and 8 mg/L as carbon of the test material was added to each flask. On day 14 pre-exposure (Day 0 of the test) the pre-exposed inoculum was coarse filtered and shaken until used. Mineral salts medium (OECD) was inoculated with 10% of the inoculum and 107 mL aliquots were dispensed into sets of 10 replicate 160 mL capacity serum bottles
Replicates:	*All groups tested in duplicate. Test groups included the following:* 1) Blanks 2) Test material at 20 mg/L carbon 3) Hexadecane at 20 mg/L carbon (reference compound) 4) Test material plus hexadecane both at 20 mg/L (inhibition control) 5) Mineral oil at 20 mg/L carbon 6) Test material at 20 mg/L carbon poisoned by addition of 50 mg/L $HgCl_2$ on day 0 and 28 (used to correct for any abiotic production of inorganic carbon).

Temperature of incubation:	20± 1°C
Study initiation:	The test material, hexadecane and mineral oil were added as measured weights on filters. The liquid to headspace ratio was 2:1. The bottles were sealed and incubated while shaking at 120 rpm. CO_2 evolution during incubation was determined by measuring inorganic carbon production. On days 7, 14, 28, 42 and 56 duplicate bottles from each group were acidified with 1 mL concentrated orthophosphoric acid, injected through the stopper, and shaken for 1 hour. The inorganic carbon concentration of the headspace was determined by duplicate injections of 1 mL headspace gas into a carbon analyzer that had been calibrated against a 1.0% w/w CO_2 in N_2 standard.
Sampling:	Days 7, 14, 28, 42 and 56
Concentration of test substance:	20 mg carbon /L
Controls:	Blank and positive controls used per guideline. Positive control was hexadecane at a loading of 20 mg carbon/L.
Method of calculating biodegradation values:	Biodegradation was calculated as net inorganic carbon (IC) production and expressed as a percentage of the theoretical maximum inorganic production (ThIC), based on the quantity of test substance (as carbon) added initially. ThIC is analogus to the term ThCO2 used in the CO2 evolution (modified Sturm) ready biodegradability test (OECD, 1992)

Results	Steady biodegradation of the test material occurred over the incubation period and mineralization of CO_2 was still occurring at the end of the test. Study Group — Mean Biodegradation at 56 Days (%ThIC) Test Material — 63 Test Material plus $HgCl_2$ (poisoned controls) — 0 Mineral Oil (Carrier Oil Control) — 65 Hexadecane (Positive Control) — 94 Test Material plus hexadecane (inhibition control) — 69 A value of $\geq$ 60% was used in this study as the pass level as this is the pass level for ready biodegradability in the CO_2 evolution test (Modified Strum Test). As the final extent of biodegradation of the test material exceeded 60% the test material was considered to have "inherent, ultimate, biodegradability". All assay validity criteria were met.
Conclusions	The test substance was considered to have "inherent, ultimate, biodegradability".
Data Quality	(1) Reliable without restriction
References	Confidential Business Information
Other	Updated: 11/21/2003

3.0 AQUATIC ORGANISMS

3.1 Acute and Prolonged Toxicity to Fish
Robust Summary 14-Fish-1

Test Substance	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts (AI-28)
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #203 Fish Acute Toxicity Test
Test Type	Semi-Static acute toxicity test (renewal)
GLP (Y/N)	Y
Year (Study Performed)	1995
Species/Strain	*Oncorhynchus mykiss*
Analytical Monitoring	Concentrations of dissolved components of the test substance were below the limit of determination (0.4 mg/L) of the analytical method.
Exposure Period (unit)	96 hours
Statistical methods	Based on the results, statistical analysis of the survival data was not performed.
Remarks field for test conditions (fill as applicable)	Fingerlings were obtained from a commercial breeder and were acclimated for nine days. The control fish had a mean length of 4.4 cm and a mean weight of 0.77 g. Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred (1 cm vortex) for 70-74 hours in a sealed 22-liter vessel. Following settling for 1-2 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study. A sealed 96 hours semi-static test was carried out with daily renewal of the test WAF's. Three 11-liter glass aspirators were filed with each WAF. A fourth chamber served as the control. Seven fish were placed in each chamber and the chambers were sealed ensuring that there was no headspace. The fish were not fed during the study. The fish were observed for toxicity at 3, 24, 48, 72 and 96 hours. At 24, 48 and 72 hours the fish were transferred to fresh WAFs or control water. Dissolved oxygen and pH were determined throughout the study. Total hardness and chlorine concentration were determined for each fresh batch of control water. Water temperature was monitored throughout the study.

Test Concentrations (Nominal)	0, 220, 460 and 1000 mg/L (Water Accommodated Fraction-WAF) Test concentrations were selected based on a range-finding study.
Results	The 24, 48, 72 and 96 hour LL_{50}'s (loading levels likely to cause 50% mortality) were all >1000 mg/L WAF.
Remarks	Range finding results indicated that the 48-hour LL_{50} was >1000 mg/L. During the main study, no toxicity was observed at dose levels up to and including 1000 mg/L (WAF). The 24, 48, 72 and 96 hour LL_{50}'s (loading levels likely to cause 50% mortality) were therefore all >1000 mg/L WAF. Water chemistry: Temperature: 16.0-16.2 ^{o}C; Dissolved Oxygen: 8.2-9.4 mg/L; pH: 7.1-7.6; Total Hardness: 266-278 mg/L as $CaCO_3$, Residual Chlorine <0.02 mg/L.
Conclusions	The 24, 48, 72 and 96 hour LL_{50}'s (loading levels likely to cause 50% mortality) were all >1000 mg/L WAF.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of test concentrations.
References	Unpublished confidential business information
Other	Updated: 11/21/2003

3.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Robust Summary 14 - Daph – 1

Test Substance	
CAS #	114959-46-5 (Test Campaign 2 AI-28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test (non-renewal)
GLP (Y/N)	Y
Year (Study Performed)	1995
Species/Strain	*Daphnia magna*
Analytical Monitoring	Concentrations of dissolved components of the test substance were below the limit of determination (0.4 mg/L) of the analytical method.
Exposure Period (unit)	48 hours
Statistical methods	Based on the results, statistical analysis of the survival data was not performed.
Remarks field for test conditions (fill as applicable)	Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred (1 cm vortex) for 47 hours in a sealed 2.25-liter vessel. Following settling for 1-1.5 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study. Twenty daphnids, less than 24 hours old were distributed into each concentration (10 daphnids/replicate). Test chambers consisted of 150 mL Erlenmeyer flasks filled with each WAF test solution. Test chambers were sealed during the study. Control test chambers were handled in an identical fashion. Daphnids were observed at 24 and 48 hours for immobility. Daphnia were considered to be immobilized if, after a brief stirring, they did not swim during a 15 second period of observation. Dissolved oxygen and pH were determined at time 0 and at 48 hours. Total hardness of control water was determined at the start of the test. Water temperature was monitored throughout the study.

Test Concentrations (Nominal)	0, 100, 220, 460 and 1000 mg/L (Water Accommodated Fraction-WAF) Test concentrations were selected based on a range-finding study.
Results	The 48 hour EL_{50} was >1000 mg/L
Remarks	Range finding results indicated that the 48 hour EL_{50} was >1000 mg/L. During the main study, no immobilization of *Daphnia magna* was observed at dose levels up to and including 1000 mg/L (WAF) at both 24 and 48 hours. The 24 and 48 hour EL_{50}'s (loading levels likely to cause 50% immobilization) were therefore >1000 mg/L WAF. Water chemistry: Temperature: 19.6-20.3^{o}C; Dissolved oxygen: 8.9-9.1 mg/L; pH: 7.9-8.2; Total Hardness: 186 mg/L as $CaCO_3$.
Conclusions	The 48 hour EL_{50} was >1000 mg/L
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of test concentrations.
References	Unpublished confidential business information
Other	Updated: 11/21/2003

Robust Summary 14-Daph– 2

Test Substance	
CAS #	114959-46-5 (test campaign AI 43)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test (non-renewal)
GLP (Y/N)	Y
Year (Study Performed)	1987
Species/Strain	*Daphnia magna*
Analytical Monitoring	Not performed
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of the survival data was not performed.
Remarks field for test conditions (fill as applicable)	Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred for 23 hours in a sealed vessel. Care was taken to ensure that emulsions did not form. Following settling for 1 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study. Thirty daphnids, less than 24 hours old were distributed into each concentration (10 daphnids/replicate). Test chambers consisted of 150 mL Pyrex crystallizing dishes containing 100 mL of test solution. Test chambers were not covered during the study. Control test chambers were handled in an identical fashion. Daphnids were observed at 24 and 48 hours for immobility and abnormal effects. Daphnia were considered to be immobilized if, after a brief stirring, they did not swim during a 10 second period of observation. Temperature, dissolved oxygen, water hardness and pH were determined at time 0 and at 48 hours. Light cycles were maintained at 16-hour light per day.
Test Concentrations (Nominal)	0, 10, 100 and 1000 mg/L (Water Accommodated Fraction-WAF)
Results	
Remarks	Immobilization of all *Daphnia magna* was observed at 1000 mg/L (WAF) at both 24 and 48 hours. Immobilization of all *Daphnia magna* was observed at 100 mg/L (WAF) at 48 hours. The no observed effect level was 10 mg/L (WAF).

	Water chemistry: Temperature: 18-22°C; Dissolved oxygen: 8.8-9.2 mg/L; pH: 8.0-8.1; Total Hardness: 170 mg/L as $CaCO_3$.
Conclusions	The 100 and 1000 mg/L (WAF) concentrations resulted in 100% immobilization at 48 hours. The 10 mg/L (WAF) concentration was the no observed effect level.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of test concentrations.
References	Unpublished confidential business information
Other	Updated: 11/21/2003

3.3 Toxicity to Aquatic Plants (e.g. Algae)

Robust Summary 14-Algae - 1

Test Substance	
CAS #	114959-46-5 (test campaign 1 AI 43)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
Method	
Method/Guideline followed	Miller, W., Green, J., The Selenastrum capricornutum algal assay bottle test (1978).
Test Type	Static acute toxicity test (Water Accommodated Fraction (WAF))
GLP (Y/N)	Not specified
Year (Study Performed)	1982
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum* derived from a strain (ATCC 22662) obtained from the American Type Culture Collection, Maryland, USA.
Element basis (# of cells/mL)	Approximately 5,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	None
Statistical methods	Mean relative growth rate for each culture was calculated.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from an in-house culture of *Pseudokirchneriella subcapitata* that was originally obtained from the American Type Culture Collection, Maryland, USA. Test System: A measured weight (100 g) of test material was added to a measured volume of distilled water (1L) and shaken for 24 hours. The supernatant was used as the stock solution. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Two 50-mL replicates per treatment, inoculum ~5,000 cells/mL. Cell counts performed on days 2 and 4. Test Levels: Control, 0.1, 0.5, 2, 10, 50, 200 and 1000 mg/L WAF loading rates. Light: Not specified.

	Test temperature: Not specified. Dilution Water: Not specified. Method of calculating mean measured concentrations: Not applicable. Exposure period: 96 hours Analytical monitoring: Not performed
Results	48-96 hour EL_{50} >1000 mg/L (WAF)
Remarks	Test Findings: At 96-hours the mean relative growth rates in the control and treated groups were as follows: WAF Concentration (mg/L) / Mean Relative Growth Rate (% of Control) 0 / - 0.1 / 103 0.5 / 100 2 / 99 10 / 97 50 / 101 200 / 101 1000 / 83 The 48-96 hour EL_{50} was greater than 1000 mg/L (WAF)
Conclusions	48-96 hour EL_{50} >1000 mg/L (WAF)
Data Quality	Reliable with restriction. Restriction due to the lack of analytical characterization of the WAF and due to the limited methodology contained in the report.
References	Confidential business information.
Other	Updated: 11/21/2003

Robust Summary 14-Algae– 2

Test Substance	
CAS #	114959-46-5 (test campaign 2 AI-28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #201 Alga Growth Inhibition Test
Test Type	Static acute toxicity test (Water Accommodated Fraction (WAF))
GLP (Y/N)	Y
Year (Study Performed)	1995
Species/Strain	Freshwater algae, *Raphidocelis subcapitata* (Nygaard) formerly called *Selenastrum capricornutum* derived from a strain (CCAP 278/4) obtained from the Institute of Freshwater Ecology, Windermere, England.
Element basis (# of cells/mL)	Approximately 5,000 cells/mL
Exposure period/duration	72 hours
Analytical monitoring	Concentrations of dissolved components of the test substance were below the limit of determination (0.4 mg/L) of the analytical method.
Statistical methods	The area under the growth curve and the average specific growth rate were determined.
Remarks field for test conditions (fill as applicable)	Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of dilution water and continuously stirred (1 cm vortex) for 45 hours in a sealed 2.3-liter vessel. Following settling for 1.5-2 hour and separation of any surface film from the water phase, the water phase was used as the test solution in the study. A 72-hour sealed static test was carried out in 287 mL full volume Erlenmeyer flasks filled with each WAF test solution. Four flasks were prepared for each of the seven WAFs along with seven control flasks containing algal growth medium only. Three out of every set of four flasks containing WAF and six of the control flasks were inoculated with 5,000 cells/mL The remaining flasks were used to determine background particle counts in the absence of Raphidocelis subcapitata. Two marbles

	were placed in each flask to ensure good mixing during incubation. Test chambers were sealed and incubated in a cool orbital incubator (100 cycles/min) under constant illumination (`4950 lux). The pH was determined at time 0 and at 72 hours. Air temperature in the test incubator was monitored throughout the study. Cell counts were made at the start of the study and then at approximately 24-hour intervals. Cell counts were made using a Coulter counter. Test Levels: Control, 10, 22, 46, 100, 220, 460 and 1000 mg/L WAF loading rates.
Results	0-72 hour EL_{50} >1000 mg/L (WAF)
Remarks	Range finding results indicated that the 72-hour EL_{50} ranged from 100 to >1000 mg/L. In the main study, culture growth was inhibited by a maximum of 22% when expressed in terms of the area under the growth curve and by 6.5% when expressed in terms of the average specific growth rate. The 72 hour EL_{50} value for both end points was therefore >1000 mg/L, the highest loading rate tested. The 24, 48 and 72 hour no observed effect loading rates (NOEL) determined for the area under the growth curve and the average specific growth rate were as follows: Exposure Period (Hours) / Area Under Growth Curve NOEL (mg/L) / Average Specific Growth Rate NOEL (mg/L) 0-24 / >1000 / >1000 0-48 / <10 / 100 0-72 / <10 / 100 The 0-48 hour and 0-72 hour NOELs of <10 mg/L were based on only 9.4% and 14% growth inhibition being identified as statistically significantly different from control. Incubation temperature: 23.3-23.8°C pH range: 7.3 (0 hour) to 9.6 (72 hours)
Conclusions	0-72 hour EL_{50} >1000 mg/L (WAF)
Data Quality	Reliable with restriction. Restriction due to the lack of analytical characterization of the WAF.
References	Confidential business information.
Other	Updated: 11/21/2003

4. Toxicity

4.1 Acute Toxicity

4.1.1 Acute Oral Toxicity

Robust Summary 14-Acute Oral –1

Test Substance	
CAS #	114959-46-5 (test campaign 2 AI - 28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Rats/Fisher 344
Sex	Male/Female
No. of animals/dose	5 /sex
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5000 mg/kg
Dose volume	4.76 mL/kg
Control group	No
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted male and female rats at a dose level of 5000 mg/kg. Clinical observations were conducted seven times on the day of test material administration and twice daily thereafter for 14 days. Individual body weights were recorded on the day of dosing, on day 8 and at termination. All animals were euthanized, and gross necropsies were performed, at the conclusion of the observation period.
Results	LD_{50} >5000 mg/kg
Remarks	All treated animal survived the 14-day duration of the study. All animals developed a hunched posture, diarrhea and an

	unkempt appearance within 2.5 hours of dosing. All rats exhibited yellow anogenital staining by day 2. All animals recovered by day 4. All animals exhibited weight gain over the 14-day observation period. No treatment related macroscopic findings were evident.
Conclusions	The test article, when administered to 5 male and 5 female rats, had an acute oral LD_{50} of >5000 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 11/21/2003

4.1.3. Acute Dermal Toxicity

Robust Summary 14-Acute Dermal –1

Test Substance	
CAS #	114959-46-5 (test campaign 2 AI 28
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Rats/Fisher 344
Sex	Male/Female
No. of animals/dose	5 /sex
Vehicle	None
Route of administration	Dermal
Dose level	2000 mg/kg
Control group	No
Chemical analysis of dosing solution	No
Remarks field for test conditions	On the day prior to dosing the dorsal fur was removed using electric clippers. On day 1 the animals were weighed and a single dose of the test material was applied to the skin. The test material was covered with a gauze dressing and waterproof adhesive tape. The animals were then individually housed. Following 24 hours the dressings were removed and the dose site washed with warm dilute detergent solution. The animals were dried and returned to group housing. Clinical observations were conducted six times on the day of test material administration and twice daily thereafter for 14 days. Individual body weights were recorded on the day of dosing, on day 8 and at termination. All animals were euthanized and gross necropsies were performed, at the conclusion of the observation period.
Results	LD_{50} >2000 mg/kg
Remarks	All treated animals survived the 14-day duration of the study.

	Female rats exhibited yellow anogenital staining from day 2. All animals recovered by day 4. Dose sites were stained brown and, in male rats, developed erythema by day 2. The treated skin was normal from day 4. All animals exhibited weight gain over the 14-day observation period. No treatment related macroscopic findings were evident.
Conclusions	The test article, when administered to 5 male and 5 female rats, had an acute dermal LD_{50} of >2000 mg/kg.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 11/21/2003

4.2 Genetic Toxicity:

Robust Summary 14-Gentox-1

Test Substance	
CAS #	CAS# 114959-46-5 (test campaign 1 AI-43)
Chemical Name	Benzoic acid, 2-hydroxy-,mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 43% active ingredient, 48% highly refined mineral oil, 9% inorganic calcium salts.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Not Specified
Year (Study Performed)	1982
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538; *Escherichia Coli* tester strains WP2 and WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	31.25, 62.5, 125, 250, 500, 1000, and 2000 ug/plate
Metabolic Activation	With and without S9 fraction mixture
Vehicle	The test material contained 38% mineral oil and was formulated in water containing 20% w/v Tween 80.
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 20.0 ug/plate TA98 - S9 benzo(a)pyrene 20.0 ug/plate TA100 +S9 benzo(a)pyrene 20.0 ug/plate TA100 - S9 benzo(a)pyrene 20.0 ug/plate TA1535 +S9 sodium azide 5.0 ug/plate TA1535 -S9 sodium azide 5.0 ug/plate TA1537 +S9 Neutral Red 20.0 ug/plate TA1537 -S9 Neutral Red 20.0 ug/plate TA1538 +S9 benzo(a)pyrene 20.0 ug/plate TA1538 - S9 benzo(a)pyrene 20.0 ug/plate WP2 +S9 4-nitroquinoline-N-oxide 20.0 ug/plate WP2 –S9 4-nitroquinoline-N-oxide 20.0 ug/plate WP2*uvr*A +S9 4-nitroquinoline-N-oxide 20.0 ug/plate WP2*uvr*A –S9 4-nitroquinoline-N-oxide 20.0 ug/plate
Vehicle Control	Vehicle control samples were prepared so that the concentration of mineral oil was comparable to that present at the highest

	concentration of the test substance. The vehicle control also contained water and Tween 80.
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strain TA100. Doses of test material ranged to 4,000 ug/plate. Cytotoxicity was evaluated.
S9 Optimization Study	Not specified
Remarks field for test conditions	This study was conducted prior to the development of OECD Guideline No. 471. This study deviates from the guideline in that Tester Strain TA 1538, not called for in the guideline, was included. The study was conducted in duplicate. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with eight concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 20 ul of test material, positive control or vehicle control were added to each plate along with each tester strain, S9 mix (if needed) and top agar. Plates were incubated for 48 hours at 37^{o}C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	No cytotoxicity was observed in the dose range finding study with tester strain TA100. Test material precipitate was not observed. In the mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. No cytotoxicity was observed up to 4,000 ug/plate with any tester strain with or without activation. The positive control for each respective test strain exhibited at least a 2.5-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.

Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 11/21/2003

Robust Summary 14-Gentox-2

Test Substance	
CAS #	114959-46-5 (test campaign 2 AI-28)
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 28% active ingredient, 51% highly refined mineral oil, 21% inorganic calcium salts
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Not Specified
Year (Study Performed)	1992
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538; *Escherichia Coli* tester strain WP2*uvr*A pKM101
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0, 31.25, 62.5, 125, 250, 500, 1000, 2000 and 5000 ug/plate
Metabolic Activation	With and without S9 fraction mixture obtained from Fisher 344 rats pretreated with Aroclor 1254
Vehicle	The test material contained mineral oil and was prepared as a formulation in 5% Tween 80 in 1:1 heptane:acetone.
Tester strain, activation status, Positive Control and concentration level	TA98 +S9 benzo(a)pyrene 10.0 ug/plate TA98 - S9 2-nitrofluorene 5.0 ug/plate TA100 +S9 benzo(a)pyrene 10.0 ug/plate TA100 - S9 sodium azide 5.0 ug/plate TA1535 +S9 2-aminoanthracene 5.0 ug/plate TA1535 - S9 sodium azide 2.0 ug/plate TA1537 +S9 neutral red 5.0 ug/plate TA1537 -S9 9-aminoacridine 5.0 ug/plate TA1538 +S9 benzo(a)pyrene 10.0 ug/plate TA1538 - S9 2-nitrofluorene 5.0 ug/plate WP2*uvr*A pKM101 +S9 benzo(a)pyrene 10.0 ug/plate WP2*uvr*A pKM101 –S9 potassium dichromate 20.0 ug/plate

Vehicle Control	5% Tween 80 in 1:1 heptane:acetone
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Not specified
S9 Optimization Study	Not specified
Remarks field for test conditions	This study was conducted prior to the development of OECD Guideline No. 471. This study deviates from the guideline in that Tester Strain TA 1538, not called for in the guideline, was included. There were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with nine concentrations of test substance, vehicle and positive controls. Three plates/dose group/strain/treatment set were evaluated. Test material (20 ul), positive control or vehicle control were added to each plate along with each tester strain, S9 mix (if needed) and top agar. Plates were incubated for 48-72 hours at 37^{o}C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	The test material formed smears on the surface of the top agar at 1000 ug/plate and above indicating that it was not miscible in the aqueous test system at these treatment levels. Microscopic examination of the background lawn showed no evidence of cytotoxicity at concentrations up to 5000 ug/plate with or without metabolic activation. In the mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. The positive control for each respective test strain exhibited at least a 7-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.

Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 11/21/2003

Robust Summary 14-Gentox-3

Test Substance	
CAS #	CAS# 114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-, mono-C14-18 alkyl derivatives, calcium salts
Remarks	Test material purity: 100%
Method	
Method/Guideline followed	OECD Guideline 473
Test Type	*In Vitro* Chromosomal Aberration Assay
GLP (Y/N)	Y
Year (Study Performed)	2005
Test System	Human peripheral blood lymphocytes
Exposure Method	Dilution
Test Substance concentration levels	Definitive Assay: 4 hour treatment, 20 hour harvest without activation: 18.75, 37.5, 75, 150, 300, 400, 500, 600 µg/mL 4 hour treatment, 20 hour harvest with activation: 12.5, 25, 50, 75, 100, 150, 200, 300 µg/mL 20 hour treatment, 0 hour harvest without activation: 6.25, 12.5, 25, 50, 75, 100 and 150 µg/mL
Metabolic Activation	With and without S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats and cofactor mix (magnesium chloride, potassium chloride, glucose-6-phosphate, NADP).
Vehicles	Water
Vehicle and Positive Control concentration levels by activation status	Mitomycin C - non-activated test system positive control (0.3 or 0.6 µg/mL) Cyclophosphamide - activated test system positive control (20 µg/mL) Water – solvent control
Statistical Analysis	Positive control groups were compared to vehicle control by Fisher Exact Test. Differences between control and treated groups were compared using Fisher Exact Test. The Cochran-Armitage assay was used to test for dose response.
Test Substance Solubility	Test substance solubility in the vehicle was determined.
Preliminary Toxicity Dose Range Finding Assay	Performed to select concentrations for the chromosome aberration assay. Consisted of an evaluation of test article effect on mitotic index. Evaluation performed at 4 hours with and without activation and at 20 hours without activation at concentrations ranging from 0.09 to 900 µg/mL.
Remarks field for test conditions	Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicle (water) was confirmed. A pretest dose range finding study was conducted at concentrations up to 900 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. Mitomycin C (positive control) was tested without activation and Cyclophosphamide (positive control) was tested with activation. Prepared cultures were treated with test substance or control material and were incubated for 4 (activated and non-activated) or 20 hours (non-activated). Two hours prior to harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.1 ug/mL. Two hours after the addition of Colcemid

	harvested cells were evaluated microscopically for percent confluency, morphology and estimated number of mitotic cells prior to harvest. Slides were prepared using Giemsa stain. Two slides/treatment group were evaluated. 200 metaphase cells (100 per culture) each containing 46 centromeres were scored. Chromosomes were counted for each cell. Chromosome aberrations, either chromosome or chromatid type were recorded. Chromatid and isochromatid gaps were presented in the data but were not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. In order for a test substance to be considered to have induced a positive response compared to vehicle control when the percentages of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. A reproducible significant increase at the high dose only with no dose response or a reproducible significant increase at one dose level other than the high dose with no dose response was considered positive. The test article was considered negative if no statistically significant increase was observed relative to the solvent control.
Results	Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.
Remarks	In the solubility evaluation water was determined to be the solvent of choice based on the partial solubility of the test material in water at a concentration of 9 mg/mL. In the pretest toxicity assay substantial toxicity was observed (at least a 50% reduction in mitotic index compared to the solvent control) at 900 and $\geq$ 90 µg/mL in the non-activated and activated 4 hour exposure groups, respectively. Substantial toxicity was observed at dose levels $\geq$ 270 µg/mL in the non-activated 20 hour group. Mitotic inhibition was 52% at 150 µg/mL in the non-activated 4-hour exposure group. The doses selected for the analysis of chromosome aberrations were 37.5, 75 and 150 µg/mL. The percentage of cells with structural or numerical aberrations in the test article groups was not significantly increased above the solvent control at any dose level. The percentage of structurally damaged cells in the positive control group was statistically significant. Mitotic inhibition was 55% at 100 µg/mL in the activated 4-hour exposure group. The doses selected for the analysis of chromosome aberrations were 12.5, 50 and 100 µg/mL. The percentage of cells with structural or numerical aberrations in the test article groups was not significantly increased above the solvent control at any dose level. The percentage of structurally damaged cells in the positive control group was statistically significant. Mitotic inhibition was 54% at 75 µg/mL in the non-activated 20-hour exposure group. The doses selected for the analysis of chromosome aberrations were

	12.5, 25 and 75 µg/mL. The percentage of cells with structural or numerical aberrations in the test article groups was not significantly increased above the solvent control at any dose level. The percentage of structurally damaged cells in the positive control group was statistically significant. Positive and vehicle control group responses were as expected. Precipitation was observed at concentrations ≥75 ug/mL in the chromosomal aberration assay. Dose kevels ≤ 50 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period.
Conclusions	Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.
Data Quality	Reliable without restriction (Klimisch Code)
References	Gudi & Rao. (2005) "In Vitro Mammalian Chromosome Aberration Test." BioReliance Study AB10VJ.341.BTL
Other	Updated: 11/18/05

4.3 Repeat Dose Toxicity

Robust Summary 14 – Repeat Dose -1

Test Substance	
CAS #	CAS# 114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-,mono-C14-18 alkyl derivs., calcium salts
Method	
Method/Guideline followed	OECD Guideline Section 407
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	2006
Species	Rat
Strain	Crl:CD (SD), Approximately 7 weeks of age at initiation of treatment
Route of administration	Oral gavage
Duration of test	28 days of treatment
Doses/concentration levels	0, 50, 150 and 500 mg/kg/day
Dose Formulation Analysis	Analysis performed for dosing solution stability, homogeneity and concentration.
Sex	Males and females
Exposure period	28-day treatment duration
Frequency of treatment	Once daily, 7 days/week
Control group and treatment	21 male and 14 female rats/group in the control and high dose group. 7 /sex/group in the low and mid dose group. Control group received daily doses of corn oil at 5.0 ml/kg, and treatment groups received the indicated dose of test material diluted in corn oil at a dose volume of 5 ml/kg
Dose Range find Study	Yes, a 14-day dose range-finding study was conducted.
Post exposure observation period	14-day recovery period in the control and high dose groups.
Statistical methods	Body weight, body weight change, food consumption, continuous functional observational battery (FOB), locomotor activity, clinical pathology and organ weight data were subjected to a parametric 1-way analysis of variance (ANOVA)) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Functional observational battery parameters that yielded scalar or descriptive data were analyzed using Fisher's Exact Test.
Remarks field for test conditions	Single oral gavage doses were administered for 28 consecutive days. Clinical examinations were performed twice daily, prior to dose administration and approximately 1 to 2 hours following dose administration. Detailed physical examinations were conducted on all animals weekly, beginning approximately 1 week prior to test article administration. Functional observational battery (FOB) assessments were recorded for 7

	animals/sex/group prior to the initiation of dose administration and during study week 3 (prior to dosing). FOB assessments were also assessed for an additional 7 males/group in the control and 500 mg/kg/day groups prior to the initiation of dose administration and during study week 3, because the males used for the initial FOB assessments were not all assigned to the 14-day recovery period. A full FOB assessment (home cage, removal from home cage handling observations, open field, sensory observations, neuromuscular observations and physiological observations) was performed. All FOB assessments were performed blind. Locomotor activity, recorded after the completion of the FOB, was measured automatically using a photobeam activity system. Individual body weights were recorded approximately weekly, beginning approximately 1 week prior to test article administration and ending on the days of the scheduled necropsies. Fasted body weights were recorded at necropsy. Individual food consumption was recorded approximately weekly, beginning approximately 1 week prior to test article administration and ending on the days of scheduled necropsies. Urinalysis, hematology, coagulation and clinical chemistry parameters were evaluated at termination of treatment and recovery from 7 animals/sex/group. Macroscopic examinations were performed on all animals. Select organs were weighed. Microscopic examination was performed on all tissues from all animals in Groups 1 and 4, sacrificed at termination of the treatment phase of the study. Gross lesions were examined from animals in Groups 2 and 3 at the primary necropsy.
Results	
Remarks	All animals survived to the scheduled necropsies. Observations of excessive salivation-related findings in the 500 mg/kg/day group males and females and at a lower incidence in the 150 mg/kg/day group males and females were noted beginning during study week 1, at the time of dosing, and at the 1-hour post-dosing observation, and persisted through the remainder of the main study. Excessive salivation-related findings were noted at 1 hour following dose administration beginning at the end of study week 0 for the additional 500 mg/kg/day males and persisted throughout the study. Observations of yellow material on various body surfaces were noted occasionally in the 500 mg/kg/day group males and females at 1 hour after dose administration near the end of the study. All other clinical findings in the test article-treated groups were noted with similar incidence in the control group, were limited to single animals, were not noted in a dose-related manner and/or were common findings for laboratory rats of this age and strain. Although not statistically significant, the mean body weights and cumulative body weight gains for the 500 mg/kg/day group males in the main study were consistently lower than the control group throughout dosing. For the additional 500 mg/kg/day group males, mean body weight gain from study week 3 to 4

was statistically significantly lower than the control group. During the recovery period, mean body weights for the 500 mg/kg/day main study group males were similar to the control group. There were no other remarkable differences when the control and test article-treated groups were compared. Food consumption was unaffected by test article administration. There were no statistically significant differences when the control and test article-treated groups were compared.

There were no test article-related effects on functional observational battery evaluations (home cage, handling, open field, sensory, neuromuscular and physiological observations) and locomotor activity. There were no test article-related alterations in urinalysis parameters.

Statistically significantly prolonged prothrombin time (↑15.8%) was present in the 500 mg/kg/day group males at the study week 4 evaluation. Although all but 1 male in this dose group had prothrombin values that were within the control group values ± 10% and while this difference in prothrombin time could be the result of normal biological variation, the possibility of a minor test article-related effect could not be ruled out. There were no other test article-related alterations in hematology and coagulation parameters.

The following alterations in serum chemistry parameters were considered related to test article administration at 500 mg/kg/day (week 4):

Parameter	**Direction and Magnitude of Change[a]**	**Sex**
Alkaline phosphatase	↑ 100.9%	Male
	↑ 197.4%	Female
Alanine Aminotransferase	↑ 65.7%	Male
	↑ 44.1%	Female
Triglyceride	↓ 43.1%	Male

a - Compared to the control group mean.

The slightly higher alkaline phosphatase and alanine aminotransferase levels at 500 mg/kg/day were associated with minimally higher liver weights (absolute and relative to body or brain weight) in males and females at this dose level. During the recovery period, the alkaline phosphatase and alanine aminotransferase levels and mean liver weights (absolute and relative to body or brain weight) were similar to the control group.

There were no test article-related gross observations at the scheduled necropsies. All macroscopic findings noted were considered to be spontaneous and/or incidental in nature and unrelated to test article administration.

The following organ weight changes were considered related to test article

administration at 500 mg/kg/day (week 4):

Parameter	Direction and Magnitude of Change[a]	Sex
Liver (absolute)	↑ 11.4%	Male
	↑ 13.9%	Female
Liver (relative to body)	↑ 15.1%	Male
	↑ 15.8%	Female
Liver (relative to brain)	↑ 14.7%	Male
	↑ 14.1%	Female
Thyroid (absolute)	↑ 46.6%	Male
Thyroid (relative to body)	↑ 60%	Male
Thyroid (relative to brain)	↑ 50.6%	Male

a - Compared to the control group mean.

There were no histological correlates to the test article-related higher organ weights. All test article-related organ weight changes subsided following the 2-week recovery period.

There were no test article-related histologic changes. All histologic changes were considered to be incidental findings, manifestations of spontaneous diseases, or related to some aspect of experimental manipulation other than administration of the test article.

Chemical analysis of dosing solutions confirmed that they were homogeneously prepared and stable at the desired concentrations for up to 10 days at refrigerated temperature. Concentration analysis confirmed that the dosing solutions were within 15% of nominal concentrations.

Conclusions	Based on the results of this study, slightly lower body weight gains (500 mg/kg/day group males), clinical findings of excessive salivation and yellow material on various body surfaces (150 and/or 500 mg/kg/day groups), slightly longer prothrombin time (500 mg/kg/day group males) and slightly higher alkaline phosphatase and alanine aminotransferase levels (500 mg/kg/day group males), with corresponding minimally higher liver weights, slightly higher thyroid weights and minimally lower triglyceride levels (500 mg/kg/day group), the no-observed-effect level (NOEL) for oral (gavage) administration of CAS #114959-46-5 to Crl:CD(SD) rats for 28 consecutive days was 50 mg/kg/day. The combined effects observed after administration of 500 mg/kg/day in males and females indicate the no-observed-adverse-effect level (NOAEL) was 150 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Kirkpatrick, J. 2006. ""A 28-day Oral (Gavage) Study of CAS# 114959-46-5 in Rats (with Functional Observational Battery and Motor Activity Determinations." WIL Study No.: 186047
Other	Updated: 10/25/2006

4.4 Reproductive and Developmental Toxicity

Robust Summary 14 – Repro/Devel – 1

Test Substance	
CAS #	114959-46-5
Chemical Name	Benzoic acid, 2-hydroxy-,mono-C14-18 alkyl derivs., calcium salts
Method	
Method/Guideline	OECD Guideline 421
Test Type	Reproduction/developmental screening study in rats
GLP (Y/N)	Y
Year (Study Performed)	2006
Species	Rat
Strain	Crl:CD(SD), approximately 62 days of age at initiation of treatment
Route of administration	Orally by gastric intubation
Duration of test	F0 males: Study Days 0-27, 14 day premating period through lactation day 3. F0 female: 14 day premating period through lactation day 3.
Dose levels	0, 50, 150 and 500 mg/kg/day
Vehicle control	Corn oil
Dose volume	5 mL/kg
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity and dose concentration confirmation. Stability of the dosing solutions was established in a prior study.
Control and treatment groups	12/sex/group
Post exposure recovery period	None
Mating ratio	One male to one female
Duration of mating period	For up to 14 days or until positive evidence of mating was observed.
Statistical methods	Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction factor. Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, offspring body weights and body weight changes, gestation length, numbers of corpora lutea, implantation sites, number of pups born, live litter size on PND 0, unaccounted-for sites, absolute and relative organ weights and pre-coital intervals were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of males at birth and postnatal survival were subjected to the Kruskal-Wallis nonparametric ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test article-treated groups to the control group.

	Histopathological findings in the test article-treated groups were compared to the control group using a two-tailed Fisher's Exact test.
Dose range finding study	Doses were selected based on prior studies conducted with this test article.
Remarks field for test conditions	Fo Generation: Viability and Toxicity: Twice daily Clinical Observations: Twice daily throughout the study. Detailed individual examinations conducted once weekly. Body Weights: F0 males: pretest, weekly through termination. F0 females: pretest, weekly during premating and mating; gestation days 0, 4, 7, 11, 14, 17 and 20; females with litters weighed on lactation days 1 and 4. Food Consumption: Pretest and weekly during treatment period. F0 males: pretest, weekly throughout the study except during mating. F0 females: pretest, weekly during premating, days 0, 4, 7, 11, 14, 17 and 20 of gestation and days 1- 4 of lactation for females with litters. Macroscopic Examinations: Performed on all animals. Organ Weights: Performed on all animals. Microscopic Examinations: Special emphasis was placed on the stages of spermatogenesis and histopathology of interstitial testicular cell structure. Microscopic examination was performed on the cervix, coagulating glands, mammary gland, thyroids with parathyroids, testes with epididymides and vas deferens, seminal vesicles, ovaries and oviducts, pituitary gland, uterus with vagina, prostate gland and all gross lesions for all animals in the control and 500 mg/kg/day groups at the scheduled necropsies; gross lesions from all dosage groups were also examined. F1 Generation: Pup/Litter Examinations: Litters observed as soon as possible after delivery for number of live and dead pups and pup abnormalities. Thereafter litters observed daily for dead pups and/or obvious irregularities. Litter Size: Recorded daily. Individual Pup Body Weights: Pup weights recorded on days 1 and 4 of lactation. Sex Determination: Days 0 and 4 of lactation. Macroscopic Examinations: Pups found dead and pups sacrificed on day 4 post-partum were carefully examined externally for gross external abnormalities, and a macroscopic examination was performed. Gross lesions and malformations were retained.
Results	Analysis of dosing solutions confirmed that the preparations were homogeneous and that they were at the appropriate concentrations. The stability of the dosing solutions was previously determined. Clinical findings of excessive pawing at the cage floor and/or walls and wiping of the mouth on the cage floor and/or walls were noted for all 12 females in the

	150 and 500 mg/kg/day groups at the time of dose administration, and for 1 and 8 females in these same groups 1-2 hours following dose administration. Salivation and related findings (consisting of clear material on various body surfaces) were noted for females in the 150 and 500 mg/kg/day groups just prior to, at the time of and/or 1-2 hours following dose administration. Excessive pawing at the cage floor and/or walls and salivation-related findings were noted to a lesser degree for males in the 500 mg/kg/day group at the time of dose administration. Salivation-related findings were also noted for males in this group just prior to and 1-2 hours following dose administration. The behavioral findings of excessive pawing of the cage floor and/or walls (males and females) and wiping of the mouth on the cage floor and/or walls (females) were likely due to test article taste aversion. Therefore, salivation-related findings noted for males and females were attributed to the test article, but were not considered adverse. Parental body weight, food consumption and reproductive parameters (e.g., mating, fertility and copulation/conception indices) were unaffected by test article administration at 50, 150 and 500 mg/kg/day. No test article-related macroscopic or microscopic findings and no adverse effects on mean organ weights were observed for males and females at any dosage level. Higher mean liver weights were noted for males (↑ 5.7%) and females (↑ 14.6%) in the 500 mg/kg/day group, but were not considered adverse, as these differences are probably consistent with hepatic enzyme induction that was adaptive in nature. Similar effects on liver weights with no morphologic correlates were reported for this test article in a previous study. F1 pups were unaffected by maternal test article administration. The mean number of pups born, live litter size on postnatal day (PND) 0, general physical condition, pup body weights and postnatal survival from birth to PND 4 were unaffected at 50, 150 and 500 mg/kg/day.
Conclusions	Because male and female reproductive parameters were unaffected by test article administration, and because no evidence of parental systemic toxicity was noted in any group, a dosage level of 500 mg/kg/day (the highest dosage level tested) was considered to be the no-observed-adverse-effect level (NOAEL) for F0 reproductive and systemic toxicity. Pup growth and survival were unaffected by the test article at all dosage levels; therefore, the NOAEL for F1 neonatal toxicity was considered to be 500 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Knapp, J. (2006) "A Reproduction/Developmental Toxicity Screening Study of CAS #114959-46-5 in Rats" WIL Research Study Number: WIL-

	186048
Other	Updated: 12/27/06